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EVALUATION OF THE TOXICITY OF MARINE SEDIMENTS AND DREDGE SPOILS WITH THE MICROTOX[R] BIOASSAY

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ABSTRACT

The Microtox[R] bioassay was used to evaluate the toxicity of sediment and dredge spoil elutriates from several potentially-contaminated sites in Mobile and Pascagoula Bays. Elutriates were prepared using either local seawater or distilled deionized water (osmotically adjusted with NaCl prior to testing), and Microtox[R] assays were performed with the elutriates and three reference toxicants. There were marked differences in the toxicity of several elutriates and reference toxicants in the two different waters, with the seawater generally resulting in the same or lesser toxicity than the osmotically-adjusted distilled deionized water.

INTRODUCTION

The Microtox[R] assay is a bacterial luminescence bioassay developed by Beckman, Inc. in 1977 as a rapid screening alternative to standard toxicity tests with fishes or invertebrates (1). The test is based on the reduction in bioluminescence of the marine bacterium, Photobacterium phosphoreum, by toxicants. The assay is simple, replicable, inexpensive and rapid. It also is relatively sensitive to many common aquatic contaminants, and has been used to assess the toxicity of a variety of aqueous samples including surface waters, leachates, effluents and different types of sediment extracts (2-13).

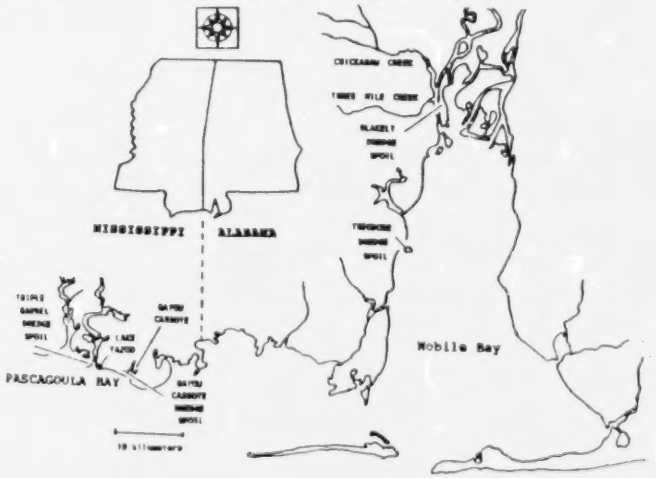
[R]Mention of products does not constitute endorsement by the U.S. Environmental Protection Agency or Fish and Wildlife Service.

Because P. phosphoreum is a marine organism, it is a logical choice for conducting toxicity studies in marine and/or estuarine environments. However, to date, the majority of work with the Microtox^{R} system has been with fresh water, rather than marine, samples. For example, Microtox^{R} has been used routinely to evaluate the toxicity of fresh water sediments (6-11); whereas relatively little work has been done with marine or estuarine sediments. Schiewe et al. (12) reported that Microtox^{R} was sensitive to toxicants extracted from marine sediments by organic solvents. Williams et al. (13) demonstrated the usefulness of Microtox^{R} for evaluating the toxicity of elutriates prepared from sediments collected from Commencement Bay, Washington. To further investigate the utility of the Microtox^{R} bioassay for marine sediment evaluation, we investigated the toxicity of several samples collected from Mobile and Pascagoula Bays, estuarine/marine systems in the southeastern U.S. Both receive pollutant inputs from a variety of point and non-point sources. Contaminants suspected to be present at high concentrations at the various sampling sites included heavy metals (Pascagoula Bay) and petroleum hydrocarbons (certain Mobile Bay sites). Extensive dredging also occurs in both areas, with the resultant potential for liberation of sediment-associated contaminants to the upper water column. To mimic the open water disposal of dredge materials, we tested the toxicity of standard sediment elutriate preparations described by the U.S. Environmental Protection Agency and the U.S. Army Corps of Engineers (14).

MATERIALS AND METHODS

Samples of sediment and dredge spoils were collected from Mobile (Alabama) and Pascagoula (Mississippi) Bays (Fig. 1 and Table 1). Aquatic sediments were collected with a Ponar grab sampler. Samples from the upland dredge spoil disposal sites were collected from 4-30 cm below the exposed soil surface, using a stainless steel spoon. Samples were

Figure 1. Sampling Locations in Mobile and Pascagoula Bays.



placed in solvent-rinsed glass jars on ice immediately upon collection, shipped to the Michigan State University Aquatic Toxicology Laboratory and held at 4°C for less than two weeks before toxicity testing.

Table 1. Sampling Sites on Mobile and Pascagoula Bay.

Site Abbreviation	Site Location
CSC-1	Chickesaw Creek (upper)
CSC-2	Chickesaw Creek (lower)
TMC	Three Mile Creek
NB	North Blakely Dredge Spoil
SB-1	South Blakely Dredge Spoil (east)
SB-2	South Blakely Dredge Spoil (west)
TD	Theodore Dredge Spoil
PTB	Pascagoula Triple Barrel Dredge Spoil
PLY	Pascagoula Lake Yazoo
PBC	Pascagoula Bayou Casotte
PBCD	Pascagoula Bayou Casotte Dredge Spoil

Elutriates were prepared using standard techniques (14). Samples were suspended in water at a ratio of 1:4 (v/v) by shaking, mixed by aeration for 30 min and centrifuged for 20 min at 6,500 g. The resultant supernatant was decanted and passed through a 1.2 micron glass wool filter before use in toxicity tests. Elutriates were prepared using both distilled deionized water (DDW) and Gulf of Mexico seawater (GSW) that had been collected approximately 100 km offshore. The salinity of the GSW was 35 g/L, as determined with a densimetric technique.

Microtox^{R} bioassays were conducted as previously described (9). Tests were performed at 15°C, in duplicate, with elutriate sample concentrations of 0, 7.5, 15, 30 and 60%. Bioluminescence was measured after 5, 15 and 30 min with a Microtox^{R} Model 2055 Toxicity Analyzer (Microbics Inc., Carlsbad, CA). Results were similar at all three time periods, so here we report only values for 15 min incubations. Osmotic strength of the samples prepared with DDW was adjusted with NaCl to 20 g/L before testing. The control/dilution water used for these samples was a 20 g/L NaCl solution. The osmotic strength of the GSW elutriate samples (35 g/L) was not further adjusted, and the control/dilution water for these samples was the GSW.

Microtox^{R} tests also were performed with three reference toxicants: copper sulfate, cadmium chloride and sodium dodecyl sulfate. Each reference compound was tested both in the GSW and the DDW (osmotically adjusted with 20 g/L NaCl).

Sample concentrations resulting in 20% inhibition of bacterial luminescence (EC_{20}) were calculated using linear regression. When EC_{20} values were greater than 100% or when the slope of the regression line was not significantly different from zero, elutriate samples were considered non-toxic.

RESULTS

Toxicity of the sediment/dredge material samples was dependent to a great extent on the type of water used to prepare the elutriates and perform the tests (Table 2). Six of the 11 samples were toxic in the DDW system; whereas only two were toxic when GSW was used. Five sites (CSC-1, NB, SB-2, PBCD, PBC) were toxic only in the DDW system, and one site (CSC-2) was toxic only in GSW. Sediments from TMC displayed similar toxicity in both types of water, and four sites (SB-1, TD, PTB, PLY) exhibited no toxicity with either type of elutriate.

Table 2. Toxicity of sediment elutriates prepared and tested in distilled deionized water (DDW) or Gulf of Mexico seawater (GSW) to Photobacterium phosphoreum. Elutriates prepared in DDW were osmotically-adjusted with NaCl (20 g/L) before testing. Values are given as EC_{20} (95% confidence interval), and are percent of sample. When EC_{20} was >100%, samples were designated as non-toxic (NT).

Site	Test Water	
	DDW	GSW
CSC-1	93.1 (89.9-96.4)	NT
CSC-2	NT	47.9 (39.0-58.9)
TMC	46.6 (35.4-61.3)	38.8 (32.2-46.7)
NB	46.7 (40.0-54.6)	NT
SB-1	NT	NT
SB-2	23.8 (18.5-30.6)	NT
TD	NT	NT
PTB	NT	NT
PLY	NT	NT
PBCD	29.2 (26.2-32.6)	NT
PBC	84.4 (58.2-122.3)	NT

The toxicity of two of the three reference compounds to P. phosphoreum also was dependent on the type of test water used (Table 3). Cadmium chloride was approximately three times more toxic in the osmotically-adjusted DDW than in GSW, whereas copper sulfate

exhibited similar toxicity in both types of water. The anionic surfactant, sodium dodecyl sulfate, was about twice as toxic in the DDW system as in GSW.

Table 3. Toxicity of three reference compounds in distilled deionized water (DDW) or Gulf of Mexico seawater (GSW) to Photobacterium phosphoreum. Samples prepared in distilled deionized water were osmotically-adjusted with NaCl (20 g/L) before testing. Values are given as EC_{20} (95% confidence interval), and are in mg/L.

	Test Water	
Toxicant	DDW	GSW
Cadmium chloride (as Cd^{++})	12.0 (10.8-13.3)	36.9 (20.4-66.7)
Copper sulfate (as Cu^{++})	0.34 (0.32-0.37)	0.28 (0.25-0.32)
Sodium dodecyl sulfate	0.45 (0.38-0.53)	0.89 (0.81-0.97)

DISCUSSION

Elutriates prepared from sediments and dredge spoils from several sites around Mobile and Pascagoula Bays exhibited slight to moderate toxicity in the Microtox^{R} bioassay; however, patterns of toxicity were dependent upon the type of water used to prepare the elutriates and perform the sample dilutions. Generally, samples prepared and tested in the GSW exhibited the same or lesser toxicity than samples prepared and tested in osmotically-adjusted DDW. This trend was observed both with elutriates of sediment samples and with the three reference toxicants.

Differences in the toxicity of elutriates made with the two types of water could have been caused by a number of factors. For example, there may have been differential extractability of contaminants associated with the sediments, such as heavy metals, during elutriate preparation, with greater concentrations partitioning into DDW than into the more ionic GSW (15). Also, the relative salinities of the two different test waters (i.e., DDW=20 g/L; GSW=35 g/L), could have influenced the bioavailability of toxic xenobiotics to the bacteria. Previous studies have established that increasing the ionic strength of test solutions markedly decreases the toxicity of metals such as zinc and cadmium, but not copper, in the Microtox^{R} bioassay (16, 17). This is in agreement with our results, and suggests that the bioavailabilities of certain metals (and perhaps other ionic compounds such as sodium dodecyl sulfate) to P. phosphoreum are affected by salinity. One other factor which could have contributed to the differential sensitivity of P. phosphoreum to elutriates and reference toxicants in the two different types of water is the physiology of

the bacteria. Because P. phosphoreum is a marine organism, it is conceivable that testing in GSW resulted in a less sensitive population than when tests were performed in osmotically-adjusted DDW.

In summary, Microtox^{R} identified sediments/dredge materials from several sites in Mobile and Pascagoula Bays as having slight to moderate toxicity; however, interpretation of patterns of toxicity was highly dependent upon the type of water used for elutriate preparation and for the assay. Therefore, when using this bioassay with marine/estuarine sediments, it is essential that careful consideration be given to a logical choice of test water. Also, based on our results with the reference toxicants, we feel that the use of Microtox^{R} with test solutions of differing osmolarity may prove to be a useful technique for establishing probable causes of observed toxicity.

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